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THE DETERMINATION OF THE ACUTE AND REPEATED ORAL TOXICITY OF HALOCARBON OIL, SERIES 27-S

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

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FOR THE COMMANDER

MBallinger

MICHAEL B. BALLINGER, Lt Col, USAF, BSC
Chief, Toxic Hazards Division
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Halocarbon 27-S (HC 27-S), a polymer of chlorotrifluoroethylene (CTFE), is used as a lubricating oil for pumps in hyperbaric chambers. Although monomeric CTFE has been shown to produce renal lesions in rats, the toxicity of CTFE polymers has not been investigated. Following a single dose of 5 g HC 27-S, no signs of toxicity were noted and no lesions observed at sacrifice 14 days following treatment. To assess the toxicity of repeated exposure to HC 27-S, three groups (n=5) of male and female Fischer-344 rats were dosed with 2.5 g HC 27-S/kg for 7 or 21 consecutive days. Groups were sacrificed at 7, 21, and 35 days after the initial dose. Decreased water consumption and urine output were apparent in all test groups. Significant increase in fluoride excretion was noted in 24-hr urine samples collected periodically during the study. The increased fluoride burden in treated animals appeared sufficient to alter bone calcium/phosphate ratios in male but not female rats. Increased liver and kidney weights were observed in all treated rats on all assessment days. Gross liver enlargement and hepatocellular cytomegaly indicated that the liver is probably the primary target organ following repeated administration of HC 27-S. Keywords; lubricants; toxicity;

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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted in the Toxic Hazards Research Unit, Northrop Services, Inc. – Environmental Sciences. This document serves as a final report on the toxicity of Halocarbon Oil, Series 27-S. The research described in this report began in December 1986 and was completed in November 1987. It was performed under U.S. Air Force Contract No. F33615-85-C-0532. Melvin E. Andersen, Ph.D., served as a Contract Technical Monitor for the U.S. Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory. The study was sponsored by the U.S. Navy under the direction of CAPT David E. Uddin, MSC, USN, and CDR David A. Macys, MSC, USN.

This work was supported by the Naval Medical Research and Development Command Task M0096-004-0006. The opinions contained herein are those of the authors and are not to be construed as official or reflecting the view of the Department of the Navy or the Naval Services at large.

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SECTION 1

INTRODUCTION

A manned hyperbaric chamber was contaminated with Halocarbon Oil, Series 27-S (HC 27-S), following the failure of a pump diaphragm. The Naval Sea Systems Command requested the Naval Medical Research Insitute, Toxicology Detachment (NMRI/TD) investigate the acute and repeated oral toxicity of HC 27-S. This was tasked to the Toxic Hazards Research Unit (THRU).

The HC 27-S is a polymer of chlorotrifluoroethylene (CTFE) and is used as a lubricating oil for pumps employed in hyperbaric chambers. In addition, HC 27-S is used to lubricate all O-rings on doors and service locks of the chambers.

Although no specific toxicity information is available on the polymers of CTFE, monomeric CTFE has been shown to produce renal damage in rats. The principal insult in the kidney was necrosis of the *pars recta*, and to a lesser extent, the *pars convoluta* of the proximal tubule. Signs of nephrotoxicity included diuresis, increased urinary lactate dehydrogenase (LDH) activity, serum creatinine, and blood urea nitrogen (BUN) with a concurrent decrease in urine osmolality. Water intake was increased by 25% in exposed rats (Potter *et al.* 1981). CTFE is metabolized to inorganic fluoride that is excreted in the urine. Plasma and urine fluoride levels in Fischer 344 (F-344) rats remained elevated for more than one week following oral exposure and for at least 24 h following inhalation exposure. Neither plasma nor urine fluoride levels was elevated following dermal exposure (Kinkead *et al.* 1987). In subchronic studies, tubules underwent regeneration and necrosis was minimal upon further exposure, suggesting adaptation to CTFE toxicity (Buckley *et al.* 1982). The authors speculated that metabolism and/or disposition of CTFE was altered, or that regenerated tissue was refractive to CTFE toxicity.

The rat was selected as the current test species to minimize space requirements and to allow comparison to the above mentioned CTFE studies. The number of rats per test group was kept to the minimum necessary for appropriate statistical analysis. These studies were performed as a preliminary assessment of the toxicity of acute and repeated exposure to HC 27-S, taking into consideration the toxicity reported for the parent monomer, CTFE.

SECTION 2

MATERIALS AND METHODS

TEST MATERIAL

The HC 27-S test material (Table 1) was supplied by NMRI/TD. The Analytical Chemistry Section of the THRU will retain an archive sample of the HC 27-S following these studies.

TABLE 1. TEST MATERIAL DATA[a]

Item	HC 27-S
MILSPEC	MIL-L-24574
Federal stock no.	9150-01-101-8835
Batch no.	86-75
NMRI/TD no.	86-295-1
Manufacturer	Halocarbon Products Corp., 82 Burlews Ct., Hackensack, NJ
Stability	Decomposes at temperatures above 260° C
Vapor pressure	Less than 0.01 mm Hg at 27° C
Additives	Organic acid rust inhibitor (0.1%)
Density[b]	1.930 g/mL at room temperature

[a] Data supplied by NMRI/TD.
[b] Data generated by THRU.

ANIMALS

F-344 rats, males weighing between 180 and 220 g and females between 150 and 200 g, were purchased from Charles River Breeding Labs of Kingston, NY. Quality control assessments, conducted during a two-week quarantine period, showed the animals to be in acceptable health.

The rats involved in the acute assay were group-housed (2-3 per cage) in plastic cages with wood chip bedding. Rats involved in the repeated assay were housed individually except during the quarantine period and the 14-day holding period postdosing. Prior to treatment the rats were put into Nalgene metabolism cages for a one-week acclimatization period. All rats were fed Purina Formulab 5008 (food in metabolism cages was powdered) and deionized distilled water *ad libitum*, with the exception that rats were fasted for 16 h prior to oral dosing. Ambient temperatures were maintained at 21-25°C except for three excursions of short duration that were caused by failures in the building's heating and air conditioning system. At those times the temperatures ranged from 13°C to 34°C. The light/dark cycle was set at 12-h intervals.

ACUTE ASSAY (ORAL LD_{50})

The rats were fasted for 16 h prior to the administration of the oral dose. Five male and five female F-344 rats were dosed with 5.0 g/kg (5.0 g of test agent per kg of body weight). The rats were weighed individually at the time of dosing to determine the dose volume of neat agent and they were maintained for 14 days of observation. The rats were weighed at 1, 4, 7, 10 and 14 days postexposure and were observed for any signs of toxicity. At the end of the 14-day observation period, all rats were sacrificed for gross pathological examination.

REPEATED ORAL ASSAY

This portion of the study involved repeated dosing at one-half the LD_{50} determined in the acute study. Since no deaths occurred at the acute limit test of 5 g/kg, the daily treatment was set at 2.5 g/kg. Six groups of six F-344 rats per sex each were dosed daily, including weekends, by gavage. Each of three test groups received the same daily dose but the groups were sacrificed at different times. Each of three control groups received an equal volume of distilled water and were sacrificed with their corresponding treatment groups (Table 2). The HC 27-S was administered as neat agent and the dose volumes were calculated from the individual body weights, adjusted daily. Dosing was performed at the beginning of each day, prior to 0930 h. Food, provided following gavage, was removed at 1630 h each day. This study was initiated using female rats, followed by an identical regimen using male rats.

TABLE 2. EXPERIMENTAL DESIGN FOR REPEATED ORAL ADMINISTRATION OF HC 27-S

Group	Dose	No. of Animals	Days on Test[a]	Days[a] at Sacrifice	Clinical Tests	Gross/ Histopathology
1	Control	6	7	8	yes	yes
2	2.5 g/kg	6	7	8	yes	yes
3	Control	6	21	22	yes	yes
4	2.5 g/kg	6	21	22	yes	yes
5	Control	6	21	36	yes	yes
6	2.5 g/kg	6	21	36	yes	yes

[a] Consecutive days including weekends.

Body weights were measured daily throughout the study. Water consumption and urine output were measured gravimetrically daily during the dosing period. Urine samples from one-day predosing, from days 1, 3, 5, 7, 14, and 21 during dosing, and from day 14 postdosing, were analyzed clinically for the parameters listed in Table 3.

TABLE 3. URINE PARAMETERS EVALUATED FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

Assay Method	Parameters
N-Multistix (Ames)	Protein Ketone Bilirubin Urobilinogen Hemoglobin pH Glucose
Refractometer	Specific gravity
DuPont ACA Picric Acid	Creatinine
Sigma Reagent Kit/and COBAS Bioassay	Serum glutamic-oxalacetic transaminase (SGOT) N-Acetyl glucose aminidase (NAG) Lactic dehydrogenase (LDH) Calcium
Fluoride Specific Electrode[a]	Inorganic fluoride

[a] Neefus *et al.* (1970).

All rats were fasted 16 h prior to sacrifice by carbon dioxide inhalation. Groups 1 and 2 were sacrificed on the morning following the seventh day of dosing; groups 3 and 4 were sacrificed on the morning following the 21st day of dosing; and groups 5 and 6 were sacrificed 14 days after the completion of dosing. At the time of sacrifice, blood was collected from the posterior *vena cava* for whole blood and serum analyses (Table 4). The following organs were weighed: heart, pituitary, liver, spleen, thymus, kidneys, testes, ovaries, and brain. A gross pathologic examination was performed on each rat, and the following specified tissues were collected and prepared for histopathologic examination. The right femur of each was collected for X-ray elemental analysis and scanning electron microscopy (SEM).

nasal turbinates	uterus[b]	kidneys
bone marrow	jejunum	prostate[a]
parathyroid	stomach	ovaries[b]
heart	colon	ileum
spleen	muscle (quadriceps, femoris)	duodenum
urinary bladder	bone (sternum and both femurs)[c]	brain
salivary glands	thyroid	nerve (sciatic)
testes[a]	lungs	esophagus
seminal vesicles[a]	liver	pituitary
pancreas	thymus	mandibular and mesenteric lymph nodes
trachea	adrenals	

[a] males only.
[b] females only.
[c] left femur for light microscopy, right femur for scanning electron microscopy and X-ray analysis.

TABLE 4. ANALYSIS OF BLOOD AND SERUM SAMPLES FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

Sample Type	Assay Method	Parameters
Whole blood	Coulter counter	White blood cell count (WBC)
		Red blood cell count (RBC)
		Hemoglobin
		Mean corpuscular volume (MCV)
		Mean corpuscular hemoglobin (MCH)
		Hematocrit
		Mean corpuscular hemoglobin concentration (MCHC)
		Differential leucocyte count
Serum	COBAS	Glucose
		Creatinine
		Blood urea nitrogen (BUN)
		Alkaline phosphatase
		Creatinine phosphokinase (CPK)
		Serum glutamic-oxalacetic transaminase (SGOT)
		Serum glutamic-pyruvic transaminase (SGPT)
		Albumin
		Total protein
		Calcium
Plasma	Fluoride Specific Electrode[a]	Inorganic fluoride

[a] Singer and Ophaug (1979).

FLUORIDE ANALYSIS

A fluoride-specific ion electrode as described by Neefus *et al.* (1970) was used to determine fluoride ion content in urine. This method utilized synthetic urine as well as a buffer for standardization. The buffer corrected for pH and provided the necessary ionic strength for linear electrode response when analyzing urine.

The method of Singer and Ophaug (1979) was used to determine unbound or ionic fluoride in plasma. A fluoride-specific electrode directly measured fluoride ion concentration following dilution of the plasma in a simple buffer system. The method was standardized using known concentrations of fluoride ion in the buffer.

X-RAY ELEMENTAL ANALYSIS

X-ray elemental analysis was conducted on the right femur of three rats of each sex per group. The femur was cleaned,(the ends removed) and fixed in 10% neutral buffered formalin for 48 h. At midshaft, the femurs were trimmed into 7-10 mm lengths the day prior to processing. The femurs were processed for SEM and X-ray analysis by dehydration with a graded series of alcohols (ethanol). The femurs were sealed into parafilm cylinders containing 100% alcohol, frozen in liquid nitrogen, and then fractured into two halves. After thawing in fresh 100% alcohol, the femurs were dried and mounted on aluminum stubs. For SEM, the fracture face was mounted in an upright position and sputter-coated with gold. The femurs were mounted on their side and a low power SEM photograph documented the site of X-ray analysis (Figure 1). Six spectra were collected from each femur in two sets of three. Spectra were collected from right to left in each set. The second set (D,E,F) was collected to the left of the first set (A,B,C). Spectra were analyzed for Ca and P using the software in a Kevex 7000 X-ray analysis system.

STATISTICAL ANALYSIS

Statistical analyses of collected data were as follows. A repeated multivariate analysis of variance with Ryan-Einot-Gabriel-Welsh F-tests was used for comparisons (Dixon 1985, Barcikowski 1983) of body weights, water consumption, urine volumes, and urine and blood chemistries between control and test groups. A two-factorial analysis of variance was performed for both sets of blood parameters, calcium-phosphorus (CaP) ratios, and organ weights (Dixon 1985, Barcikowski 1983). Histopathologic data were analyzed using the Yates's corrected Chi-square test (Zar 1974). A probability of 0.05 or less denoted a significant statistical change from controls.

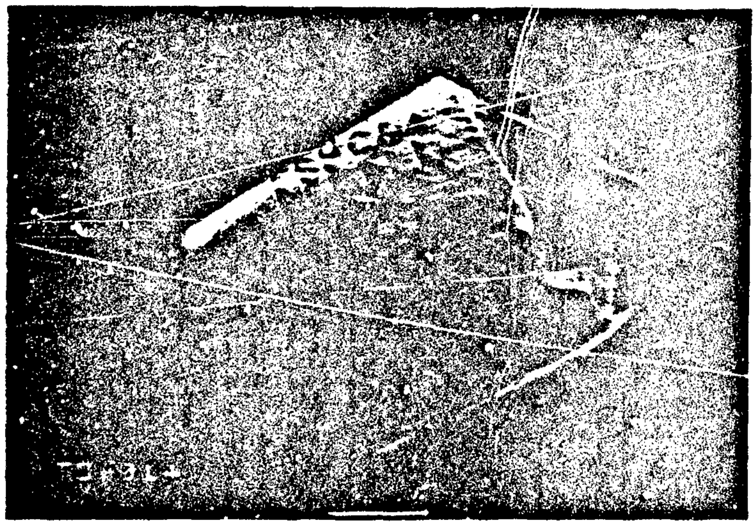

Figure 1. Sites of X-ray Analysis on Rat Femur Following Repeated Oral Administration of HC 27-S.

SECTION 3

RESULTS

ACUTE ORAL TOXICITY

Groups of five male and five female F-344 rats were orally dosed with 5.0 g HC 27-S/kg body weight. During the 14-day observation period, all animals gained weight and showed no signs of clinical toxicity. Gross pathological examination of the male rats at the conclusion of the 14-day observation period revealed no gross lesions. Four of the five female rats exhibited a bilateral petechial hemorrhage of the thymus. (The body weights from these studies have been provided in Appendix A.

REPEATED ORAL ASSAY

Three test and two control male rats and four test female rats died during the repeated dosing regimen prior to scheduled sacrifice. Two test and one control male rat and two of four female rats had sufficient gavage trauma as to have been the cause of death. Tonoclonic spasms were noted in the female test rats after 4 day's treatment and were noted sporadically throughout the rest of the study. Convulsions were observed in four different rats on three separate occasions. Not all female rats were observed to have convulsed, nor was a single rat observed to have convulsed more than once. Test subjects of both sexes had mild diarrhea by 5 to 6 days and appeared lethargic by 8 to 10 days. The female rats appeared unkempt and kyphotic by 11 days into the study. A few treated rats had dried blood and/or hematoporphyrin-like residue around the mouth and nares. Convulsions were not noted in male rats at any time throughout the study.

Both sexes of rats dosed with HC 27-S had lower mean body weights than their respective control group at most weighing dates (Figures 2 and 3). Following an early depression in the mean body weight of the male test group (days 1 and 3), no difference from the controls was noted until day 17, after which the mean body weights of the test group were significantly ($p < 0.01$) depressed throughout the remainder of the study (Table 5). The mean body weight of the test female group was generally lower than the control group but the difference was statistically significant only on days 3 through 17 (Table 6).

The mean daily water consumption of treated animals was consistently less throughout the study. The decrease in daily water consumption of the treated male rats (Table 7) was significantly less ($p < 0.01$) at 2 of the first 6 treatment days, then remained so throughout most of the 21-day treatment period. Overall the treated male rats consumed 17% less water than their respective controls. The mean daily water consumption of the test female rats (Table 8) was consistently less

< 0.01) than their respective controls throughout the study. The test female rats consumed 25% less water than the controls.

Commensurate with the decrease in daily water consumption was a decrease in urine output in both sexes of rats (Tables 9 and 10). Urine output by both male and female test animals was generally depressed (p < 0.01) throughout the treatment period. Based on the days when urine volumes were measured, treated male rats produced 75% the urine volume of control males while the treated female group produced only 54% the urine volume of control females.

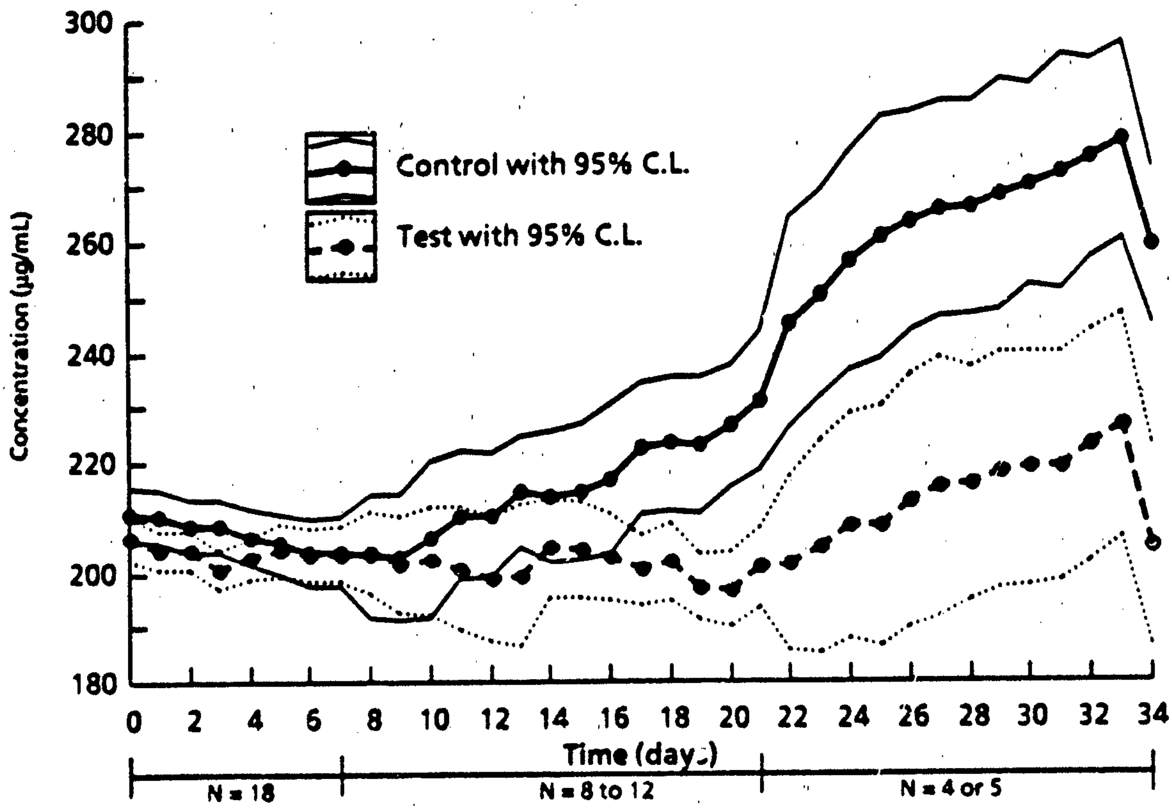

Figure 2. Mean Body Weights of Male F-344 Rats Following Repeated Oral Administration of HC 27-S. Test animal body weights were statistically different (P<0.01) from controls on days 1, 3 and days 17 through 34.

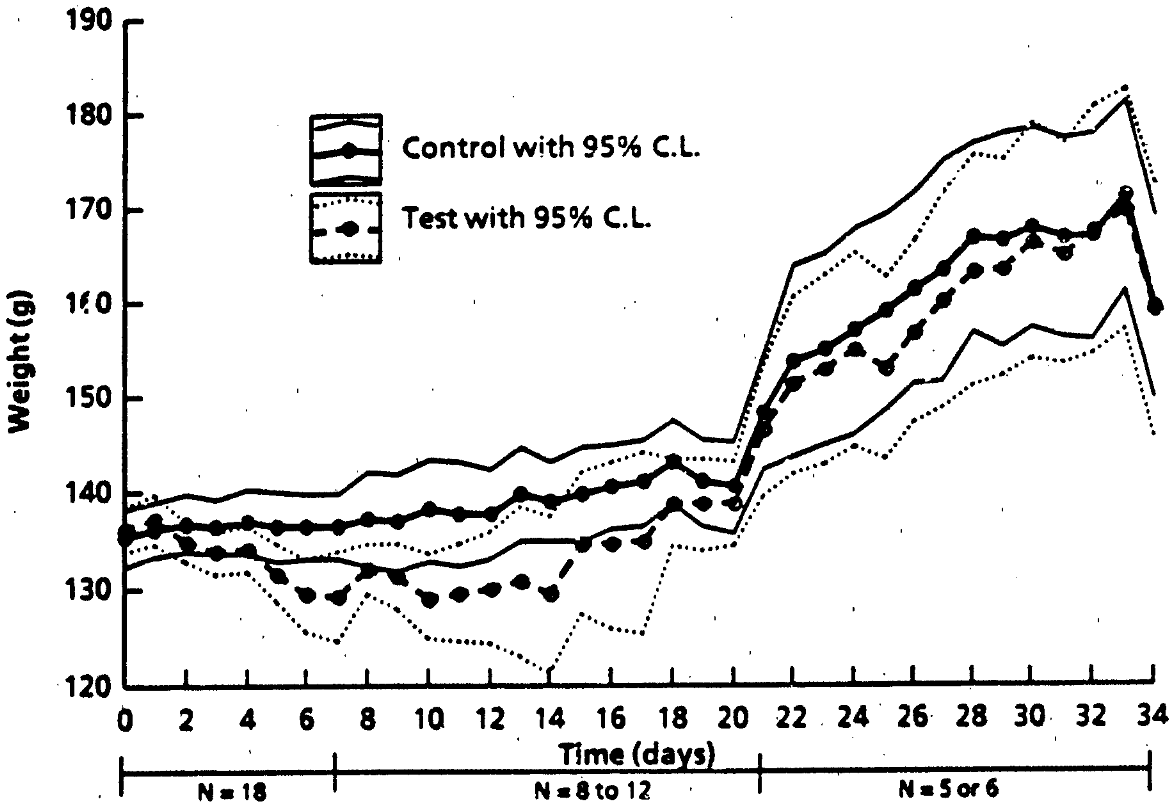

Figure 3. **Mean Body Weights of Female F-344 Rats Following Repeated Oral Administration of HC 27-S. Test animal body weights were statistically different ($P<0.05$) from controls on days 3 through 17.**

The urine clinical parameters analyzed periodically during the study were generally within normal limits. Those parameters that demonstrated statistically significant differences are listed in Tables 11 and 12. The only parameter that indicated an apparent treatment-related effect was creatinine which denoted an increase after a single treatment followed by a gradual return toward control values. Although several urine-specific gravity and pH measurements were found to be different ($p < 0.05$) from control values, all were within the normal range expected for rats and are not of apparent biological significance. The results of the urine biochemistry analyses are listed in Tables 13 and 14. Again, a number of test parameters are noted as significantly different from control values. However, except for LDH and SGOT values in female rats, the control values are inconsistent and the differences noted do not appear to represent treatment-related effects. The SGOT activity of the test female rats was higher ($P < 0.01$) at five of the seven time points assessed during the treatment period. The SGOT activity appeared to return toward control values during the 14-day posttreatment period (Table 14). The LDH concentrations in the urine of both control and treated female rats generally increased throughout the study. However, increase with time was more marked in the treated rats as the LDH content of the urine was almost twice (189%) that of the controls at the 35-day sacrifice.

TABLE 5. MEAN[a] BODY WEIGHTS (g) OF MALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

Day	Controls	Test
0	211 ± 2.4 (18)	206 ± 1.8 (18)
1	210 ± 2.3 (18)	204 ± 1.7 (18)[b]
2	209 ± 2.3 (18)	204 ± 1.7 (18)
3	209 ± 2.3 (18)	201 ± 1.6 (18)[b]
4	207 ± 2.3 (18)	202 ± 1.8 (17)
5	205 ± 2.6 (18)	204 ± 2.2 (17)
6	204 ± 3.0 (18)	203 ± 2.3 (17)
7	204 ± 3.1 (18)	204 ± 2.6 (16)
8	203 ± 5.0 (12)	204 ± 3.4 (11)
9	203 ± 5.2 (12)	201 ± 4.0 (11)
10	205 ± 6.1 (12)	201 ± 4.8 (11)
11	210 ± 5.3 (11)	201 ± 5.0 (11)
12	210 ± 5.0 (11)	199 ± 5.2 (11)
13	214 ± 4.6 (11)	200 ± 5.8 (11)
14	214 ± 5.4 (11)	204 ± 4.0 (10)
15	215 ± 5.5 (11)	204 ± 3.9 (10)
16	217 ± 6.2 (11)	203 ± 3.4 (10)
17	223 ± 5.2 (10)	201 ± 2.9 (10)[b]
18	223 ± 5.3 (10)	202 ± 3.1 (10)[b]
19	223 ± 5.4 (10)	197 ± 2.6 (10)[b]
20	227 ± 4.9 (10)	197 ± 3.1 (10)[b]
21	231 ± 5.5 (10)	201 ± 3.2 (10)[b]
22	245 ± 6.0 (4)	201 ± 5.7 (5)[b]
23	251 ± 5.9 (4)	205 ± 7.0 (5)[b]
24	257 ± 6.2 (4)	208 ± 7.3 (5)[b]
25	261 ± 6.8 (4)	208 ± 7.8 (5)[b]
26	264 ± 6.2 (4)	213 ± 8.2 (5)[b]
27	266 ± 6.1 (4)	215 ± 8.5 (5)[b]
28	266 ± 6.1 (4)	216 ± 7.6 (5)[b]
29	268 ± 6.5 (4)	218 ± 7.7 (5)[b]
30	270 ± 5.7 (4)	219 ± 7.7 (5)[b]
31	272 ± 6.7 (4)	219 ± 7.4 (5)[b]
32	275 ± 5.6 (4)	223 ± 7.5 (5)[b]
33	278 ± 5.5 (4)	227 ± 7.3 (5)[b]
34	259 ± 4.4 (4)[c]	205 ± 6.6 (5)[b,c]

[a] Mean ± SEM (N).
[b] Statistically different from controls $p<0.01$.
[c] Fasted weights.

TABLE 6. MEAN[a] BODY WEIGHTS (g) OF FEMALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

Day	Controls	Test
0	135 ± 1.4 (18)	136 ± 1.1 (18)
1	136 ± 1.4 (18)	137 ± 1.2 (18)
2	137 ± 1.4 (18)	135 ± 1.0 (18)
3	136 ± 1.4 (18)	134 ± 1.0 (18)[c]
4	139 ± 1.5 (18)	134 ± 1.1 (18)[c]
5	136 ± 1.7 (18)	132 ± 1.3 (18)[b]
6	136 ± 1.6 (18)	129 ± 1.8 (18)[b]
7	136 ± 1.5 (18)	129 ± 2.2 (18)[b]
8	137 ± 2.2 (12)	132 ± 1.2 (12)[c]
9	137 ± 2.2 (12)	131 ± 1.5 (12)[b]
10	138 ± 2.4 (12)	129 ± 2.0 (12)[b]
11	138 ± 2.4 (12)	130 ± 2.3 (12)[b]
12	138 ± 2.1 (12)	130 ± 2.6 (11)[b]
13	140 ± 2.2 (12)	131 ± 3.5 (11)[b]
14	139 ± 1.9 (12)	129 ± 3.6 (11)[b]
15	140 ± 2.2 (12)	135 ± 3.2 (10)[c]
16	140 ± 2.0 (12)	135 ± 3.7 (9)[b]
17	141 ± 2.0 (12)	135 ± 4.0 (9)[b]
18	143 ± 2.0 (12)	139 ± 1.9 (8)
19	141 ± 2.0 (12)	139 ± 2.0 (8)
20	140 ± 2.1 (12)	139 ± 1.8 (8)
21	148 ± 2.7 (12)	146 ± 2.9 (8)
22	154 ± 3.9 (6)	151 ± 3.4 (5)
23	155 ± 4.0 (6)	153 ± 3.6 (5)
24	157 ± 4.2 (6)	155 ± 3.7 (5)
25	159 ± 4.0 (6)	155 ± 4.0 (5)
26	161 ± 3.9 (6)	157 ± 3.4 (5)
27	163 ± 4.5 (6)	160 ± 4.0 (5)
28	167 ± 3.9 (6)	163 ± 4.4 (5)
29	166 ± 4.4 (6)	163 ± 4.1 (5)
30	168 ± 4.1 (6)	166 ± 4.5 (5)
31	167 ± 4.1 (6)	165 ± 4.2 (5)
32	167 ± 4.3 (6)	167 ± 4.7 (5)
33	171 ± 3.9 (6)	170 ± 4.6 (5)
34[d]	159 ± 3.7 (6)[d]	159 ± 4.8 (5)[d]

[a] Mean ± SEM (N)
[b] Statistically different from controls $p < 0.01$
[c] Statistically different from controls $p < 0.05$
[d] Fasted weights.

TABLE 7. DAILY WATER CONSUMPTION[a] (g) OF MALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

Day	Controls	Test
0	22.0 ± 0.8 (18)	21.7 ± 0.5 (18)
1	23.5 ± 0.5 (18)	16.8 ± 1.0 (18)[b]
2	19.9 ± 1.6 (18)	22.8 ± 0.8 (18)[c]
3	24.2 ± 0.8 (18)	16.5 ± 1.4 (18)[b]
4	19.2 ± 1.8 (18)	22.0 ± 0.9 (17)[c]
5	17.7 ± 1.8 (18)	19.1 ± 1.3 (17)
6	17.8 ± 1.8 (18)	16.9 ± 1.4 (17)
7	20.9 ± 1.8 (18)	16.5 ± 1.5 (17)
8	18.3 ± 1.8 (12)	19.2 ± 1.0 (11)
9	19.2 ± 2.0 (12)	14.4 ± 1.7 (11)
10	21.8 ± 1.9 (12)	16.5 ± 2.1 (11)
11	21.4 ± 1.0 (11)	15.4 ± 1.7 (11)[b]
12	22.5 ± 0.8 (11)	16.5 ± 1.3 (11)[b]
13	24.4 ± 0.6 (11)	16.8 ± 1.5 (11)[b]
14	22.1 ± 1.9 (11)	20.6 ± 0.8 (10)
15	22.6 ± 1.5 (11)	19.1 ± 0.7 (10)
16	22.3 ± 2.1 (11)	16.2 ± 1.1 (10)[b]
17	22.8 ± 0.6 (10)	16.4 ± 1.1 (10)[b]
18	22.0 ± 1.7 (10)	19.1 ± 1.1 (10)
19	23.6 ± 0.9 (10)	15.5 ± 1.5 (10)[b]
20	22.2 ± 0.7 (10)	17.8 ± 1.4 (10)[b]
21	23.6 ± 1.4 (10)	21.1 ± 0.9 (10)
35	13.4 ± 1.8 (4)[d]	4.5 ± 2.0 (5)[b,d]

[a] Mean ± SEM (N).
[b] Statistically different from controls $p<0.01$.
[c] Statistically different from controls $p<0.05$.
[d] Animals fasted.

TABLE 8. DAILY WATER CONSUMPTION[a] (g) OF FEMALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

Day	Controls	Test
0	21.7 ± 1.7 (18)	18.3 ± 0.6 (18)
1	21.6 ± 1.3 (18)	18.4 ± 0.5 (18)[c]
2	17.7 ± 1.2 (18)	9.8 ± 0.7 (18)[b]
3	15.7 ± 1.0 (18)	11.5 ± 0.6 (18)[b]
4	17.9 ± 1.0 (18)	14.1 ± 0.7 (18)[b]
5	16.2 ± 1.3 (18)	9.0 ± 1.0 (18)[b]
6	17.8 ± 1.1 (18)	10.3 ± 0.9 (18)[b]
7	14.8 ± 0.8 (18)	11.1 ± 0.9 (18)[b]
8	18.1 ± 0.7 (12)	12.5 ± 0.6 (12)[b]
9	14.3 ± 0.8 (12)	10.4 ± 1.3 (12)[b]
10	17.1 ± 0.6 (12)	9.2 ± 1.7 (12)[b]
11	15.2 ± 1.2 (12)	11.1 ± 1.3 (12)
12	15.3 ± 1.1 (12)	9.3 ± 1.3 (12)[b]
13	16.5 ± 0.9 (12)	11.0 ± 1.5 (11)[b]
14	12.4 ± 1.6 (12)	9.3 ± 1.4 (11)
15	16.4 ± 1.0 (12)	13.8 ± 1.3 (10)
16	16.1 ± 1.0 (12)	12.5 ± 0.8 (9)[b]
17	15.1 ± 0.8 (12)	11.6 ± 1.1 (9)[b]
18	15.2 ± 0.7 (12)	11.4 ± 1.3 (8)[b]
19	13.7 ± 1.5 (12)	11.3 ± 0.9 (8)[b]
20	15.2 ± 1.3 (12)	13.1 ± 0.8 (8)
21	19.4 ± 1.3 (12)	16.9 ± 1.9 (8)
35	20.0 ± 1.7 (6)[d]	13.7 ± 1.4 (5)[b,d]

[a] Mean ± SEM (N).
[b] Statistically different from controls $p<0.01$.
[c] Statistically different from controls $p<0.05$.
[d] Animals fasted.

TABLE 9. DAILY URINE OUTPUT[a] (g) OF MALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

Day	Controls	Test
0	10.0 ± 0.5 (18)	10.6 ± 0.6 (18)
1	10.5 ± 0.5 (18)	6.8 ± 0.4 (18)[b]
2	9.6 ± 0.8 (18)	9.4 ± 0.6 (18)
3	11.1 ± 0.7 (18)	7.8 ± 0.6 (18)[b]
4	10.6 ± 1.0 (18)	9.5 ± 0.7 (17)
5	9.1 ± 0.8 (18)	8.7 ± 0.6 (17)
6	9.8 ± 1.0 (18)	8.4 ± 0.8 (17)
7	10.1 ± 1.0 (18)	6.9 ± 0.6 (16)[b]
8	10.1 ± 0.9 (12)	7.7 ± 0.6 (11)[b]
9	10.5 ± 1.2 (12)	7.1 ± 0.8 (11)[b]
10	11.9 ± 1.0 (12)	7.3 ± 0.8 (11)[b]
11	11.8 ± 0.9 (11)	7.2 ± 0.8 (11)[b]
12	12.5 ± 0.7 (11)	7.8 ± 0.8 (11)[b]
13	13.6 ± 0.5 (11)	8.7 ± 0.7 (11)[b]
14	12.9 ± 0.5 (11)	9.8 ± 0.6 (10)[b]
15	11.8 ± 1.1 (11)	9.1 ± 0.5 (10)[b]
16	11.3 ± 1.0 (11)	8.0 ± 0.6 (10)[b]
17	12.4 ± 0.5 (10)	8.7 ± 0.6 (10)[b]
18	12.0 ± 0.9 (10)	9.0 ± 0.7 (10)[b]
19	13.3 ± 0.7 (10)	9.4 ± 0.9 (10)[b]
20	11.5 ± 0.6 (10)	9.7 ± 0.6 (10)[b]
21	10.9 ± 0.6 (10)	8.8 ± 0.6 (10)[b]
35	13.9 ± 2.4 (4)[c]	8.5 ± 1.7 (5)[c]

[a] Mean ± SEM (N).
[b] Statistically different from controls $p < 0.01$
[c] Animals fasted.

TABLE 10. DAILY URINE OUTPUT[a] (g) OF FEMALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

Day	Controls	Test
0	11.0 ± 1.6 (18)	8.5 ± 0.6 (18)
1	10.4 ± 1.1 (18)	6.6 ± 0.4 (18)
2	10.1 ± 1.0 (18)	4.1 ± 0.4 (18)[b]
3	8.8 ± 0.8 (18)	4.4 ± 0.4 (18)[b]
4	7.8 ± 0.8 (18)	4.1 ± 0.4 (18)
5	10.5 ± 0.9 (18)	4.2 ± 0.4 (18)[b]
6	9.3 ± 0.8 (18)	4.0 ± 0.5 (18)[c]
7	7.8 ± 0.4 (18)	3.9 ± 0.3 (17)[b]
8	9.2 ± 0.5 (12)	4.7 ± 0.5 (12)[b]
9	7.5 ± 0.6 (12)	4.6 ± 0.6 (12)[b]
10	9.7 ± 0.7 (12)	4.3 ± 0.6 (12)[b]
11	9.8 ± 0.9 (12)	4.8 ± 0.7 (12)[b]
12	8.9 ± 0.8 (12)	3.7 ± 0.6 (12)[b]
13	9.4 ± 0.7 (12)	5.3 ± 0.8 (11)[b]
14	8.2 ± 0.9 (12)	5.1 ± 0.7 (11)[b]
15	10.8 ± 1.4 (6)[d]	5.9 ± 0.7 (6)[b,d]
16	9.8 ± 0.7 (12)	6.1 ± 0.7 (9)[b]
17	8.6 ± 0.7 (12)	5.7 ± 0.7 (9)[b]
18	8.0 ± 0.5 (12)	4.8 ± 0.3 (8)[b]
19	8.6 ± 1.1 (12)	4.3 ± 0.5 (8)[b]
20	8.4 ± 1.0 (12)	5.1 ± 0.7 (8)[b]
21	8.4 ± 0.5 (12)	5.8 ± 0.4 (8)[b]
35	12.3 ± 2.1 (6)[e]	5.7 ± 1.1 (5)[b,e]

[a] Mean ± SEM (N).
[b] Statistically different from controls $p<0.01$.
[c] Statistically different from controls $p<0.05$.
[d] Some samples were inadvertently not measured.
[e] Animals fasted.

TABLE 11. MEAN[a] URINE CHEMISTRY VALUES FOR MALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

	Specific Gravity	
Day	Control	Test
0	1.014 ± .002 (18)	1.016 ± .002 (18)
1	1.010 ± .001 (18)	1.014 ± .001 (18)
3	1.046 ± .002 (18)	1.048 ± .002 (18)
5	1.051 ± .001 (18)	1.054 ± .001 (17)
7	1.051 ± .003 (18)	1.058 ± .002 (17)[b]
14	1.039 ± .002 (11)	1.043 ± .002 (10)
21	1.048 ± .003 (10)	1.054 ± .002 (10)
35	1.030 ± .003 (4)	1.052 ± .004 (5)[b]

	pH	
Day	Control	Test
0	7.3 ± 0.1 (18)	7.5 ± 0.1 (18)
1	8.1 ± 0.1 (18)	7.4 ± 0.1 (18)[b]
3	8.2 ± 0.1 (18)	7.9 ± 0.1 (18)[b]
5	7.9 ± 0.1 (18)	7.9 ± 0.1 (17)
7	7.9 ± 0.1 (18)	7.8 ± 0.1 (17)
14	7.9 ± 0.1 (11)	7.9 ± 0.1 (10)
21	8.0 ± 0.0 (10)	7.9 ± 0.1 (10)
35	7.5 ± 0.2 (4)	6.7 ± 0.2 (5)[c]

	Creatinine (mg/dL)	
Day	Control	Test
0	81.1 ± 4.5 (17)	74.6 ± 3.1 (18)
1	86.5 ± 3.2 (18)	121.7 ± 4.8 (18)[b]
3	95.9 ± 4.5 (18)	117.7 ± 8.0 (18)[b]
5	101.8 ± 5.8 (18)	100.8 ± 6.0 (17)
7	97.9 ± 7.0 (18)	118.4 ± 6.5 (17)[b]
14	77.9 ± 1.7 (11)	76.9 ± 3.6 (10)
21	97.6 ± 5.4 (10)	81.9 ± 3.8 (10)[b]
35	92.7 ± 3.7 (4)	88.8 ± 11.1 (5)

[a] Mean ± SEM (N)
[b] Statistically different from controls $p < 0.01$.
[c] Statistically different from controls $p < 0.05$.

TABLE 12. MEAN[a] URINE CHEMISTRY VALUES FOR FEMALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

	Specific Gravity	
Day	Control	Test
0	1.008 ± .001 (18)	1.008 ± .001 (18)
1	1.009 ± .001 (18)	1.009 ± .001 (18)
3	1.009 ± .001 (18)	1.012 ± .001 (18)[b]
5	1.010 ± .001 (18)	1.012 ± .002 (13)
7	1.010 ± .001 (18)	1.014 ± .002 (17)
14	1.011 ± .001 (12)	1.014 ± .002 (10)
21	1.010 ± .001 (12)	1.011 ± .001 (8)
35	1.012 ± .002 (6)	1.015 ± .003 (5)

	pH	
Day	Control	Test
0	8.0 ± 0.1 (18)	8.0 ± 0.1 (18)
1	8.2 ± 0.1 (18)	8.2 ± 0.1 (18)
3	8.1 ± 0.1 (18)	8.0 ± 0.1 (18)
5	8.0 ± 0.1 (18)	8.0 ± 0.2 (13)
7	7.9 ± 0.1 (18)	7.7 ± 0.2 (17)
14	8.0 ± 0.1 (12)	7.9 ± 0.2 (10)
21	7.8 ± 0.1 (12)	7.9 ± 0.2 (8)
35	7.3 ± 0.2 (6)	7.1 ± 0.3 (5)

	Creatinine (mg/dL)	
Day	Control	Test
0	68.2 ± 6.0 (18)	75.4 ± 3.6 (18)
1	66.1 ± 5.4 (18)	85.5 ± 4.3 (18)[b]
3	84.3 ± 8.8 (18)	123.7 ± 6.8 (18)[b]
5	61.9 ± 4.7 (18)	103.4 ± 8.2 (13)[b]
7	73.3 ± 3.1 (18)	108.4 ± 6.4 (18)[b]
14	82.3 ± 8.4 (12)	95.0 ± 8.4 (10)
21	79.1 ± 4.6 (12)	94.4 ± 5.6 (8)[b]
35	65.7 ± 15.5 (6)	109.8 ± 19.2 (4)

[a] Mean ± SEM (N).
[b] Statistically different from controls $p<0.01$.

TABLE 13. MEAN VALUE[a] OF URINE BIOCHEMISTRY PARAMETERS[b] OF MALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

Day	SGOT (U/L) Control	Test
0	11.3 ± 0.8 (18)	10.1 ± 0.7 (18)
1	11.4 ± 1.0 (18)	31.8 ± 18.1 (18)
3	9.4 ± 0.7 (18)	15.9 ± 2.1 (17)[c]
5	9.6 ± 1.0 (18)	12.9 ± 2.0 (17)
7	11.5 ± 2.3 (17)	18.5 ± 4.0 (17)
14	11.5 ± 0.8 (11)	13.8 ± 1.6 (10)
21	8.9 ± 2.4 (10)	10.8 ± 1.4 (10)
35	9.6 ± 1.7 (4)	8.3 ± 0.5 (5)

Day	NAG (U/L) Control	Test
0	45.3 ± 4.4 (18)	39.0 ± 4.0 (18)
1	23.6 ± 1.2 (18)	58.3 ± 9.4 (18)[c]
3	28.0 ± 1.8 (18)	44.9 ± 5.3 (18)[d]
5	32.4 ± 3.6 (18)	40.4 ± 3.2 (17)
7	34.6 ± 6.7 (17)	46.5 ± 8.8 (17)
14	18.3 ± 1.9 (11)	29.0 ± 4.4 (10)[c]
35	1.3 ± 0.2 (4)	3.7 ± 0.6 (5)[c]

Day	LDH (µg/mL) Control	Test
0	14.3 ± 1.4 (18)	13.6 ± 1.8 (15)
1	14.4 ± 0.9 (17)	41.8 ± 18.5 (17)
3	7.5 ± 1.0 (16)	17.9 ± 2.5 (15)[c]
5	8.4 ± 1.4 (17)	16.2 ± 1.6 (15)[c]
7	15.9 ± 2.3 (18)	29.0 ± 7.5 (17)
14	13.8 ± 0.9 (11)	12.3 ± 0.9 (10)
21	16.4 ± 1.4 (10)	13.5 ± 1.5 (10)
35	10.6 ± 1.6 (4)	16.4 ± 4.5 (5)

Day	Calcium (mg/dL) Control	Test
0	1.6 ± 0.1 (18)	1.6 ± 0.1 (18)
1	1.7 ± 0.1 (18)	1.9 ± 0.1 (18)
3	3.9 ± 0.4 (18)	2.1 ± 0.1 (18)
5	6.5 ± 0.9 (18)	5.0 ± 0.6 (17)
7	8.9 ± 1.2 (18)	5.0 ± 0.8 (16)[c]
14	12.9 ± 1.5 (11)	2.9 ± 0.3 (10)[c]
21	14.2 ± 1.2 (10)	5.4 ± 0.6 (10)[c]
35	8.7 ± 0.4 (4)	9.7 ± 0.6 (5)

[a] Mean ± SEM (N).
[b] Data provided by NMRI/TD.
[c] Statistically different from controls $p < 0.01$.
[d] Statistically different from controls $p < 0.05$.

TABLE 14. MEAN VALUES[a] OF URINE BIOCHEMISTRY PARAMETERS[b] FOR FEMALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

Day	SGOT (U/L) Control	SGOT (U/L) Test
0	3.6 ± 0.4 (16)	5.0 ± 0.7 (18)[c]
1	2.8 ± 0.3 (18)	5.6 ± 0.7 (18)[c]
3	3.2 ± 0.4 (18)	12.4 ± 2.6 (18)
5	4.1 ± 0.5 (18)	14.7 ± 4.3 (13)[c]
7	3.2 ± 0.5 (18)	17.4 ± 4.2 (17)[c]
14	7.7 ± 3.0 (12)	12.8 ± 2.1 (10)
21	3.9 ± 0.6 (12)	9.9 ± 1.1 (8)[c]
35	3.3 ± 0.5 (6)	6.2 ± 1.9 (5)

Day	NAG (U/L) Control	NAG (U/L) Test
0	13.7 ± 1.7 (16)	18.5 ± 1.5 (18)[c]
1	17.4 ± 1.4 (18)	19.7 ± 1.4 (18)
3	22.1 ± 2.0 (18)	42.9 ± 4.3 (18)[c]
5	20.3 ± 2.0 (18)	44.2 ± 7.6 (13)
7	27.1 ± 1.7 (18)	76.0 ± 15.2 (17)
14	20.3 ± 3.2 (12)	61.3 ± 21.2 (11)[c]
21	25.5 ± 1.9 (12)	54.6 ± 9.4 (8)[c]
35	46.3 ± 14.3 (6)	68.6 ± 19.2 (5)

Day	LDH (µg/mL) Control	LDH (µg/mL) Test
0	7.3 ± 1.1 (14)	8.9 ± 0.8 (18)
1	7.3 ± 1.0 (18)	11.3 ± 0.8 (18)[c]
3	8.2 ± 1.4 (16)	16.9 ± 2.3 (18)
5	9.3 ± 1.2 (17)	17.4 ± 2.5 (13)[c]
7	11.1 ± 0.7 (18)	19.5 ± 1.5 (17)[c]
14	12.6 ± 1.7 (12)	13.3 ± 2.2 (11)
21	11.6 ± 1.2 (12)	15.2 ± 1.5 (7)
35	10.9 ± 2.6 (6)	20.6 ± 4.8 (5)[c]

Day	Calcium (mg/dL) Control	Calcium (mg/dL) Test
0	8.5 ± 0.7 (16)	8.6 ± 0.7 (18)
1	8.9 ± 0.5 (18)	8.5 ± 0.5 (18)
3	16.6 ± 2.3 (18)	6.8 ± 0.8 (18)[c]
5	15.9 ± 0.9 (18)	7.0 ± 0.7 (13)[c]
7	14.9 ± 0.8 (18)	4.6 ± 0.4 (17)[c]
14	13.1 ± 1.0 (12)	4.4 ± 0.4 (11)[c]
21	15.6 ± 0.7 (12)	6.1 ± 0.9 (8)[c]
35	17.6 ± 3.5 (6)	7.6 ± 2.3 (5)[c]

[a] Mean ± SEM (N).
[b] Data provided by NMRI/TD.
[c] Statistically different from controls $p < 0.01$

The results of serum biochemistry evaluations are listed in Tables 15 and 16. There is a significant decrease in glucose values for the test male rats at each evaluation period while test female rats had increased ($p < 0.01$) glucose concentrations at 21 and 35 days. Both sexes had increased ($p < 0.05$) albumin concentrations at 21 and 35 days, but only the females had a commensurate increase in total protein at the same evaluation periods. Several parameters noted as significant in male rats were outside normal ranges and appear to be treatment-related. BUN concentrations were 28 and 78% greater ($p < 0.05$) than control concentrations at 21 and 35 days, respectively. Serum concentrations of alkaline phosphatase of the treated animals progressively increased ($p < 0.01$) by 35, 179, and 216% greater than control concentrations at each of the evaluation periods. Albumin concentrations were also higher ($p < 0.05$) at each of the evaluation periods; however, the increases above control values were not as dramatic (8, 25, and 21%, respectively, at the 7-, 21-, and 35-day evaluations).

Lymphocytosis was a common finding in both sexes of test rats sacrificed at 21 and 35 days. The statistically significant ($p < 0.05$) increase in the numbers of lymphocytes was commensurate with a similar decrease in the number of neutrophils. The results of the whole blood evaluations are presented in Appendices B and C.

Inorganic fluoride concentrations of 24-h urine collections, measured periodically during the study (Figure 4), increased throughout the treatment period, especially during the first 7-day treatment period. The fluoride concentrations in the urine returned toward baseline or control levels following treatment; however, complete recovery was not achieved by 14-days posttreatment. The increase in urine fluoride concentrations was statistically significant after a single treatment in both male and female rats (Table 17). Plasma inorganic fluoride concentrations were measured at each sacrifice. The 7- and 21-day sacrifices occurred 24 h after the final oral dose of HC 27-S while the 35-day sacrifice occurred 14 days following the final dose. Plasma inorganic fluoride concentrations were not elevated at any of these time periods (Table 18); in fact, the plasma fluoride concentrations of the test rats were less than that of the controls at five of the six evaluation periods.

X-ray elemental analysis was performed on femurs from three rats per sex, per group following sacrifice to determine if deposition of fluoride in the bone matrix was sufficient to alter CaP ratios. (CaP data for individual rat femurs are presented in Appendices D through I.) Treated male rats had CaP ratios that were significantly greater than the ratios of the control male rats at each sacrifice period (Table 19). No CaP differences were found between treated and control female rats at any of the sacrifice periods.

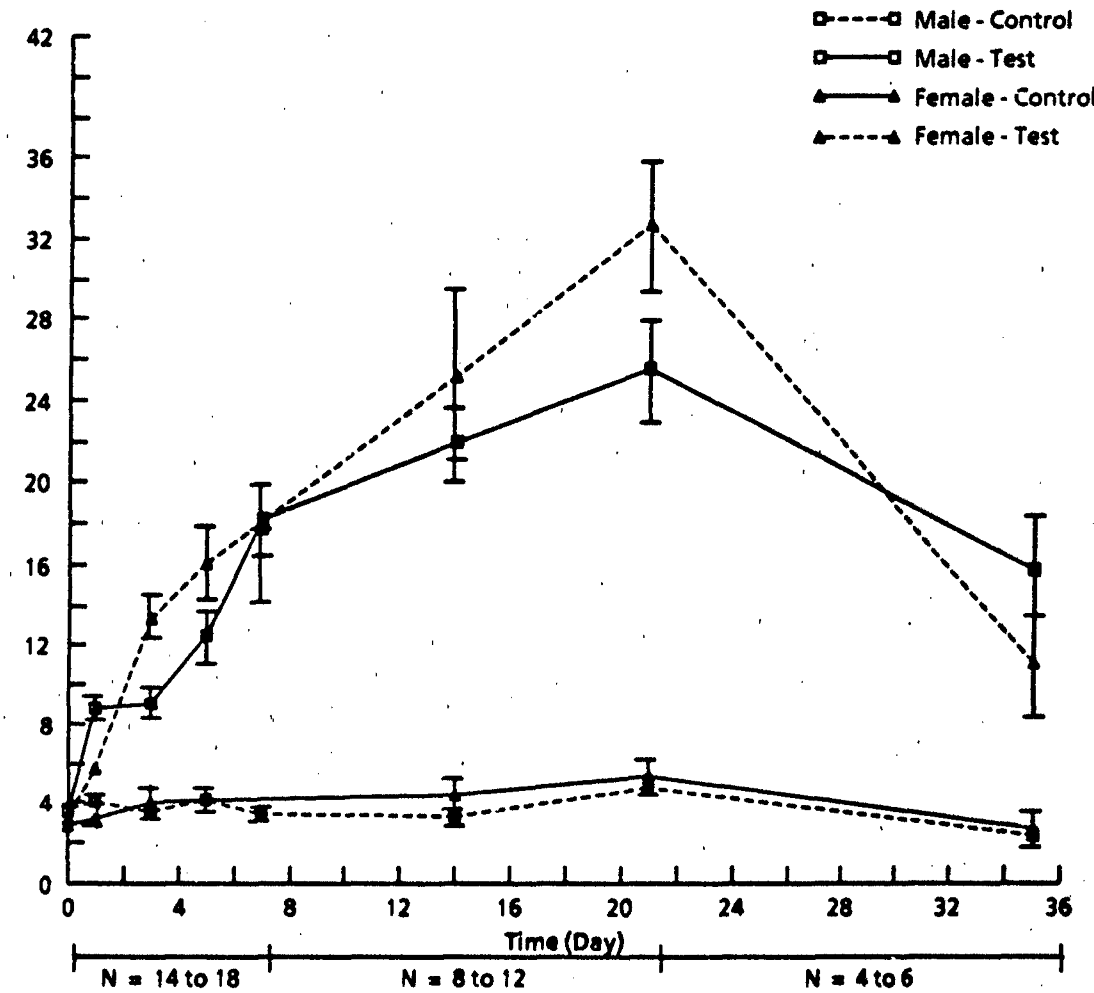

Figure 4. Urine Fluoride Concentration of F-344 Rats Following Repeated Oral Administration of HC 27-S for 21 Days. Test animal urine fluoride concentrations were statistically different ($P<0.01$) from controls on days 1 through 35.

Although a number of the organ weight means and organ-to-body weight ratio means were statistically different ($p < 0.05$) from the controls (Tables 20 and 21), no remarkable trends were noted for these organs and the changes were not considered treatment-related. Notable increases in relative kidney weights of the treated rats of both sexes occurred at each of the sacrifice periods. The increased kidney-to-body weight ratio of female rats remained consistent throughout the study while the ratio in male rats continued to increase during the treatment and posttreatment periods. Mean liver weights of the treated rats were markedly increased over controls except at the initial sacrifice (7 days) of female rats. Relative liver weights of the treated male rats were increased over controls by 70, 175, and 180% at the 7-, 21-, and 35-day sacrifice periods, respectively, and those of the treated female rats were increased over controls by 35, 125, and 65% at the respective sacrifice

periods. The relative liver weights of treated male rats continued to increase between sacrifice days 21 and 35 even though treatment with HC 27-S had been terminated. An increase ($p < 0.05$) in the relative testes weights of the treated male rats was noted at the 21- and 35-day sacrifice periods. However, the absolute weight of the testes increased at rates comparable to that of controls throughout the study.

Gross pathologic findings in rats at the conclusion of each treatment period consisted of enlarged livers and adrenal glands in both sexes. Enlarged mesenteric lymph nodes were noted in female rats killed after 7 days. Generalized atrophy of the thymus was noted in a number of the male rats killed at 21 and 35 days.

Significant microscopic changes were restricted to the organs of deglutition (pharynx/larynx and esophagus), mandibular lymph nodes, thoracic cavity, liver, adrenal glands, and possibly the kidneys. Most lesions in the organs of deglutition and thoracic cavity were compatible with gavage trauma. Moderate to severe acute/chronic inflammation and hemorrhage were observed in the larynx, esophagus, and thoracic cavity of rats of both sexes from all treatment groups. Mild to moderate hepatocellular swellings were present in 100% of the male rats treated with HC 27-S (Table 22). Concurrently, 67, 100, and 83% ne treated females sacrificed following 7, 21, and 35 days, respectively, exhibited hepatocellular swelling. When graded on a severity scale of 0-4 (0 = no effect, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe), the following averages were established for hepatocellular swelling in each treatment group. Control rats did not display this lesion and are not included.

Time of Sacrifice	Males	Females
7 Days	2.7	1.0
21 Days	3.0	2.5
35 Days	2.8	1.2

Minimum to mild vacuolar degeneration (diagnosed as fatty change) of adrenocortical cells was present in 100% of the treated males and 50% of the treated females killed following the 21-day treatment. Similarly, 67% of the males and 83% of the females killed following the 14-day recovery period also exhibited mild adrenocortical vacuolar changes. Adrenocortical changes were noted in only one control rat from the study.

Although increased weights were reported in kidneys harvested from treated rats, microscopic findings were generally unremarkable. Four of six treated male rats killed at 7 days displayed mild accumulations of hyaline droplets (resorbed protein) in the cytoplasm of proximal tubular epithelial cells. Similar hyaline droplets were not recorded in males killed at 21 or 35 days.

TABLE 15. MEAN VALUES[a] OF SERUM BIOCHEMISTRY PARAMETERS[b] FOR MALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

	7 Days	21 Days	35 Days
Glucose (mg/dL)			
Control	169.0 ± 14.9	207.7 ± 16.0	154.4 ± 18.9
Test	116.1 ± 9.0[c]	125.8 ± 4.7[c]	132.3 ± 5.7[c]
Creatinine (mg/dL)			
Control	0.7 ± 0.0	1.1 ± 0.1	0.5 ± 0.0
Test	0.7 ± 0.0	1.1 ± 0.0	0.4 ± 0.0
BUN (mg/dL)			
Control	17.9 ± 1.0	14.0 ± 0.9	16.9 ± 0.4
Test	16.5 ± 0.3	18.1 ± 1.0[d]	30.1 ± 0.7[c]
Alk. Phos. (IU/L)			
Control	115.3 ± 4.9	118.1 ± 6.3	65.3 ± 4.0
Test	155.6 ± 6.3[c]	329.3 ± 18.2[c]	206.5 ± 26.1[c]
CPK (IU/L)			
Control	94.3 ± 34.6	57.5 ± 1.5	410.2 ± 343.8
Test	86.4 ± 17.6	49.2 ± 2.3	269.8 ± 205.3
SGOT (IU/L)			
Control	48.2 ± 2.4	45.9 ± 1.2	56.9 ± 4.8
Test	54.9 ± 2.1[d]	56.3 ± 5.1[d]	64.1 ± 13.1[d]
SGPT (IU/L)			
Control	33.3 ± 2.4	30.9 ± 1.8	39.9 ± 2.1
Test	52.9 ± 6.2[c]	51.6 ± 7.1[c]	45.7 ± 1.6[c]
Albumin (g/dL)			
Control	3.9 ± 0.0	3.9 ± 0.0	5.2 ± 0.1
Test	4.2 ± 0.1[d]	4.9 ± 0.1[d]	6.2 ± 0.2[d]
Total Protein (g/dL)			
Control	6.1 ± 0.1	6.3 ± 0.1	6.4 ± 0.2
Test	6.1 ± 0.1	6.9 ± 0.2	6.6 ± 0.1
Calcium (mg/dL)			
Control	12.2 ± 0.2	10.2 ± 0.2	10.5 ± 0.1
Test	11.8 ± 0.4	10.5 ± 0.2	9.6 ± 0.6

[a] Mean ± SEM
[b] Data provided by NMRI/TD.
[c] Statistically different from control $p < 0.01$.
[d] Statistically different from control $p < 0.05$.

TABLE 16. MEAN VALUES[a] OF SERUM BIOCHEMISTRY PARAMETERS[b] FOR FEMALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

	7 Days	21 Days	35 Days
Glucose (mg/dL)			
Control	71.1 ± 4.7	109.4 ± 5.3	92.2 ± 1.8
Test	79.2 ± 5.4	149.5 ± 7.7[c]	103.0 ± 7.6[c]
Creatinine (mg/dL)			
Control	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0
Test	0.4 ± 0.0[c]	0.7 ± 0.0	0.6 ± 0.0
BUN (mg/dL)			
Control	21.9 ± 0.5	21.6 ± 0.5	15.8 ± 0.6
Test	21.7 ± 5.2	19.1 ± 1.3	16.6 ± 2.1
Alk. Phos. (IU/L)			
Control	90.9 ± 2.4	94.2 ± 3.9	73.0 ± 3.7
Test	84.3 ± 3.7	98.7 ± 4.8	129.3 ± 6.7[c]
CPK (IU/L)			
Control	93.0 ± 8.4	54.2 ± 2.1	151.6 ± 29.4
Test	197.2 ± 35.0[c]	51.1 ± 3.5	174.0 ± 6.4
SGOT (IU/L)			
Control	72.4 ± 5.7	51.2 ± 2.2	61.1 ± 1.4
Test	70.1 ± 3.3	47.8 ± 1.6	51.3 ± 2.0
SGPT (IU/L)			
Control	53.2 ± 3.3	31.6 ± 1.4	34.3 ± 2.8
Test	41.6 ± 4.2	33.0 ± 0.9	32.8 ± 0.9
Albumin (g/dL)			
Control	3.9 ± 0.0	3.8 ± 0.0	3.7 ± 0.0
Test	3.5 ± 0.1	4.3 ± 0.1[c]	4.4 ± 0.2[c]
Total Protein (g/dL)			
Control	6.2 ± 0.1	6.1 ± 0.1	5.8 ± 0.1
Test	5.8 ± 0.2	6.9 ± 0.2[c]	7.1 ± 0.1[c]
Calcium (mg/dL)			
Control	10.7 ± 0.1	10.3 ± 0.1	9.7 ± 0.1
Test	11.4 ± 0.4	10.5 ± 0.1	10.0 ± 0.3

[a] Mean ± SEM
[b] Data provided by NMRI/TD.
[c] Statistically different from control $p < 0.01$.

TABLE 17. URINARY FLUORIDE CONCENTRATIONS[a] (mg/L) FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

Day	MALE RATS	
	Control	Test
0	3.69 ± 0.19 (18)	3.55 ± 0.18 (18)
1	4.04 ± 0.16 (18)	8.78 ± 0.42 (18)[b]
3	3.55 ± 0.20 (18)	9.07 ± 0.62 (17)[b]
5	4.17 ± 0.23 (18)	12.47 ± 1.15 (17)[b]
7	3.42 ± 0.23 (18)	18.20 ± 1.60 (17)[b]
14	3.30 ± 0.26 (11)	21.99 ± 1.79 (10)[b]
21	4.78 ± 0.27 (10)	25.54 ± 2.35 (10)[b]
35	2.34 ± 0.25 (4)	15.78 ± 2.35 (5)[b]

Day	FEMALE RATS	
	Control	Test
0	2.84 ± 0.24 (18)	3.18 ± 0.18 (18)
1	3.26 ± 0.30 (17)	5.67 ± 0.35 (18)[b]
3	4.03 ± 0.36 (18)	13.38 ± 0.90 (18)[b]
5	4.12 ± 0.39 (18)	16.12 ± 1.58 (14)[b]
7[c]		
14	4.47 ± 0.52 (12)	25.21 ± 3.97 (11)[b]
21	5.40 ± 0.55 (12)	32.67 ± 3.14 (8)[b]
35	2.72 ± 0.66 (6)	11.06 ± 2.57 (5)[b]

[a] Mean ± SEM (N).
[b] Statistically different from controls $p<0.01$.
[c] Data unavailable due to instrument malfunction.

TABLE 18. PLASMA FLUORIDE CONCENTRATIONS OF RATS AT SACRIFICE FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

Sex/Group	Fluoride Concentrations[a], mg/L		
	7 Days	21 Days	35 Days
Males			
Control	0.20 ± 0.03 (6)	0.46 ± 0.08 (5)	0.75 ± 0.05 (3)
Test	0.16 ± 0.02 (5)	0.56 ± 0.09 (5)	0.45 ± 0.06 (5)[b]
Females			
Control	0.29 ± 0.02 (6)	0.31 ± 0.09 (3)	0.37 ± 0.05 (3)
Test	0.23 ± 0.03 (6)	0.23 ± 0.01 (3)	0.23 ± 0.01 (5)[b]

[a] Mean ± SEM (N).
[b] Statistically different from controls $p<0.01$.

TABLE 19. MEAN[a] CaP MEASUREMENTS OF FEMURS FROM RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S (N = 3)

Sex/Group	7 Days	21 Days	35 Days
Males			
Control	1.63 ± 0.02	1.64 ± 0.02	1.55 ± 0.03
Test	2.05 ± 0.02[b]	1.96 ± 0.02[b]	1.87 ± 0.06[b]
Females			
Control	1.63 ± 0.02	1.59 ± 0.01	1.68 ± 0.01
Test	1.66 ± 0.03	1.66 ± 0.02	1.68 ± 0.03

[a] Mean ± SEM (N).
[b] Statistically different from controls $p<0.01$.

TABLE 20. MEAN ORGAN WEIGHTS[a] (g) AND ORGAN TO BODY WEIGHT RATIOS OF MALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

	7 Days		21 Days		35 Days	
	Control (6)	Test (5)	Control (6)	Test (5)	Control (6)	Test (5)
Brain	1.77 ± .01	1.77 ± .02	1.77 ± .02	1.79 ± .00	1.91 ± .01	1.88 ± .01
Ratio[b]	0.87 ± .02	0.85 ± .01	0.80 ± .02	0.88 ± .01	0.74 ± .01	0.92 ± .03[c]
Thymus	0.23 ± .01	0.23 ± .01	0.26 ± .01	0.19 ± .01[c]	0.29 ± .01	0.21 ± .02[d]
Ratio	0.11 ± .01	0.11 ± .00	0.12 ± .00	0.09 ± .00	0.11 ± .01	0.10 ± .01
Heart	0.73 ± .02	0.71 ± .01	0.81 ± .03	0.72 ± .02	0.97 ± .02	0.76 ± .03[c]
Ratio	0.36 ± .01	0.34 ± .01	0.37 ± .01	0.35 ± .01	0.37 ± .01	0.37 ± .02
Liver	6.60 ± .42	11.51 ± .33[c]	7.23 ± .41	17.90 ± .33[c]	8.08 ± .17	17.90 ± .69[c]
Ratio	3.21 ± .15	5.53 ± .12[c]	3.25 ± .11	8.80 ± .21[c]	3.12 ± .04	8.73 ± .12[c]
Spleen	0.45 ± .02	0.49 ± .01	0.48 ± .02	0.47 ± .02	0.66 ± .01	0.49 ± .02[c]
Ratio	0.22 ± .01	0.24 ± .01	0.22 ± .01	0.23 ± .01	0.25 ± .01	0.24 ± .01
Kidney	1.64 ± .08	1.95 ± .04	1.78 ± .06	2.08 ± .02	2.07 ± .03	2.35 ± .10
Ratio	0.80 ± .03	0.94 ± .02[c]	0.80 ± .01	1.02 ± .01[c]	0.80 ± .01	1.15 ± .02[c]
Testes	2.80 ± .04	2.78 ± .03	2.75 ± .02	2.76 ± .05	2.93 ± .04	2.90 ± .06
Ratio	1.37 ± .02	1.34 ± .03	1.24 ± .03	1.36 ± .02[d]	1.13 ± .02	1.42 ± .03[c]
Whole Body	204.5 ± 4.1	206.5 ± 2.0	222.2 ± 6.3	203.6 ± 3.1	259.3 ± 4.4	204.8 ± 6.5[c]

[a] Mean ± SEM (N).
[b] Organ weight/body weight x 100.
[c] Statistically different from controls at $p<0.01$ level.
[d] Statistically different from controls at $p<0.05$ level.

TABLE 21. MEAN ORGAN WEIGHTS[a] (g) AND ORGAN TO BODY WEIGHT RATIOS OF FEMALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

	7 Days		21 Days		35 Days	
	Control (6)	Test (6)	Control (6)	Test (3)	Control (6)	Test (5)
Brain	1.64 ± .02	1.66 ± .02[d]	1.64 ± .01	1.71 ± .00[d]	1.69 ± .02	1.71 ± .02[d]
Ratio[b]	1.20 ± .02	1.35 ± .06[d]	1.15 ± .03	1.19 ± .04	1.06 ± .02	1.08 ± .03
Thymus	0.23 ± .01	0.21 ± .03	0.23 ± .01	0.22 ± .01	0.25 ± .02	0.25 ± .02
Ratio	0.17 ± .01	0.17 ± .02	0.16 ± .01	0.15 ± .01	0.16 ± .01	0.16 ± .01
Heart	0.56 ± .02	0.50 ± .03	0.52 ± .01	0.50 ± .01	0.63 ± .02	0.67 ± .02
Ratio	0.41 ± .02	0.40 ± .02	0.37 ± .01	0.35 ± .02	0.40 ± .01	0.42 ± .01
Liver	4.04 ± .12	5.16 ± .38	4.03 ± .10	8.96 ± .12[d]	4.66 ± .18	7.59 ± .47[d]
Ratio	2.96 ± .05	4.14 ± .18[c]	2.81 ± .06	6.26 ± .29[c]	2.92 ± .07	4.81 ± .40[c]
Spleen	0.35 ± .01	0.32 ± .03	0.39 ± .02	0.42 ± .01	0.45 ± .01	0.50 ± .01
Ratio	0.26 ± .01	0.25 ± .02	0.27 ± .01	0.30 ± .00	0.28 ± .01	0.31 ± .01
Kidney	1.05 ± .03	1.11 ± .04	1.10 ± .03	1.29 ± .01[d]	1.26 ± .04	1.47 ± .03[d]
Ratio	0.77 ± .01	0.90 ± .03[c]	0.77 ± .01	0.90 ± .04[c]	0.79 ± .01	0.93 ± .04[c]
Ovary	0.10 ± .01	0.09 ± .01	0.10 ± .01	0.09 ± .01	0.12 ± .01	0.11 ± .01
Ratio	0.07 ± .01	0.07 ± .00	0.07 ± .01	0.06 ± .01	0.07 ± .01	0.07 ± .01
Whole Body	136.7 ± 2.8	123.7 ± 5.4	143.3 ± 2.5	143.7 ± 5.5	159.3 ± 3.7	159.0 ± 4.8

[a] Mean ± SEM (N).
[b] Organ weight/body weight x 100.
[c] Statistically different from controls at $p<0.01$ level.
[d] Statistically different from controls at $p<0.05$ level.

TABLE 22. INCIDENCE (%) SUMMARY OF SELECTED MICROSCOPIC LESIONS OF RATS FOLLOWING REPEATED ADMINISTRATION OF HC 27-S

	Treatment Group											
	7 Day				21 Day				35 Day			
	Male		Female		Male		Female		Male		Female	
Organ: Lesion	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Adrenal:												
Fatty Change	0	17[a]	0	0	0	100[a]	0	50[a]	17	67[a]	0	83[a]
Liver:												
Hepato-cellular Swelling	0	100[a]	0	67[a]	0	100[a]	0	100[a]	0	100[a]	0	83[a]
Kidneys:												
Hyaline Droplets	0	67	0	0	0	0	0	0	17	0	0	0
Mesenteric LN:												
Lymphocytic Hyperplasia	0	0	0	0	17	17	0	0	20	0	0	0
Mandibular LN:												
Lymphocytic Hyperplasia	0	0	0	0	50	33	17	0	67	50	0	0
Thymus:												
Atrophy	17	0	0	0	0	0	0	0	0	0	0	0

[a] Statistically different from controls $p<0.01$.

SECTION 4

DISCUSSION

Repeated oral dosing of HC 27-S to male and female rats resulted in unthrifty hair coat, lethargy, and diarrhea. Convulsions occurred as early as four days and continued irregularly thereafter in the female rats. All of these toxic signs are typical of acute fluoride poisoning (Haynes and Murad 1985).

Depression of body weight gains throughout the first 21 days of the study appears to be related to treatment and housing (metabolism cages with ground food and a daily 16-h fast period) even though the rats had a one-week acclimation period prior to the treatment period. Following the 21-day treatment period the rats were returned to shoebox cages, pelletized food, and unfasted conditions. All groups except the male test group showed a dramatic increase in mean body weights during the subsequent 14 days. In addition to the loss of weight, as the study progressed the rats became increasingly irritable and difficult to handle, resulting in a number of cases of gavage trauma.

The results of clinical analyses of urine samples indicated an increase in creatinine excretion during the first week of the study. Creatinine is normally excreted through the kidneys in quantities proportional to serum content (Widmann 1973). Serum creatinine values for the test rats at the 7-day sacrifice (when urine creatinine values were elevated) indicated no significant increase above control values. In addition, there were no significant increases in serum creatinine values at the 21- or 35-day sacrifices. The significance of the increased values and whether either is indicative of kidney damage remain questionable.

The elevated concentrations of serum BUN in the male rats indicate that plasma clearance ability may have been affected. Return toward normal values was not apparent following the 14-day posttreatment period. The elevated serum albumin levels in both sexes of treated rats and elevated total protein in female treated rats may be related to reduced water consumption and resultant dehydration. No explanation can be rendered for glucose values of treated rats that were significantly different from those of control rats since the trends were opposite for each sex of treated rats.

The major route of fluoride excretion is by the kidneys (Haynes and Murad 1985) and was obvious in this study as daily levels of inorganic fluoride in the urine of the test rats significantly increased throughout the study. The mean concentration of inorganic fluoride in the urine of the test rats examined following 14 and 21 days of treatment ranged between 22 and 32 mg/L,

approximately six to seven times that of the respective controls. Fourteen days following cessation of treatment, the test rats were still excreting between 11 and 16 mg inorganic fluoride per liter, compared with control rats that excreted between 2 and 6 mg/L throughout the study. It is reported in Patty's Industrial Hygiene and Toxicology (1963) that the mean concentration of inorganic fluoride in the urine of Danish cryolite workers who complained of loss of appetite, shortness of breath, and nausea was 16.05 mg/L (range of 2.41 to 43.41). In those workers with less severe exposure, the mean urinary concentration of fluoride was 4.81 mg/L (range of 1.78 to 11.67). It was also reported that heavily exposed aluminum workers (aluminum is produced by the electrolysis of bauxite in a bath of molten cryolite) had a mean daily urine fluoride concentration of 9.03 mg/L. In two factories in the United States, increases in the radiographic density of the bones have appeared in men whose urine was known to have contained ≥ 10 mg inorganic fluoride per liter.

Plasma inorganic fluoride concentrations measured at 7, 21, and 35 days were not elevated when compared with controls. Previous studies with CTFE oligomers of shorter chain length (Kinkead *et al.* 1987) reported significantly elevated plasma fluoride 7 days after a single oral dose. However, in this study the blood samples of the test animals were, at five of the six examination times, lower in fluoride concentrations than the control animals. It appeared as though an overcompensation occurred in the HC 27-S-treated rats, resulting in abnormally lower plasma fluoride levels, or levels which peaked at less than 24 h.

Previous investigators (Clayton *et al.* 1977, Potter *et al.* 1981, Buckley *et al.* 1982) reported increased water uptake and urine output in rats treated with or exposed to CTFE. The increases were accompanied by significant decreases in urinary osmolalities. Rats treated with HC 27-S exhibited the opposite effects, with significant decreases in both water consumption and urine output and no appreciable change in urine-concentrating ability.

The CaP ratios obtained in this study were from the mineralized matrix of rat femurs below the periosteum. This area of bone should reflect any changes in bone metabolism or mineralization due to its proximity to the periosteum, the area of rapid bone turnover. The CaP ratios were in the normal physiological range of 1.3 to 2.0 (Guyton 1976) for all but one group of male rats. The ratio greater than 2.0 (2.05 ± 0.02 SEM) from the male group treated for 7 days appeared to peak at 7 days, then leveled out over the 21 days of treatment, and slowly decreased after cessation of treatment. Since bone turnover in rats is rapid, this is not surprising. In identically treated female rats, CaP ratios did not vary from control female CaP ratios.

The activity of serum alkaline phosphatase was elevated in the treated male rats at each sacrifice period. A slight increase in serum alkaline phosphatase activity in treated female rats was observed only at the final sacrifice. The most likely explanation for the higher enzyme activity in

male rats is their more severe hepatocytomegalic liver disease. Enlarged hepatocytes probably compressed canaliculi and small biliary ducts causing partial intrahepatic cholestasis. Bile flow obstruction very commonly leads to the induction of alkaline phosphatase synthesis in the liver. Correspondingly, SGOT and SGPT, derived from injured hepatocytes, become elevated in the blood, as occurred in the HC 27-S-treated male rats. Since the rats used in the study were relatively young, their relatively high rates of bone osteoblastic activity may have contributed to the serum alkaline phosphatase level. However, the bone contribution was probably insignificant since the microscopic examination failed to disclose altered bone structure in either treated or control male rats. The slightly higher serum alkaline phosphatase activities of male controls as compared to female controls are reflective of the faster growth rate of the males as compared to females.

Since fluoride is incorporated into the bone by replacing hydroxyapatite with the denser fluoroapatite (calcium fluoride, Haynes and Murad 1985), it is reasonable to expect that the CaP ratio will increase as fluoride replaces phosphorus in the bone. Fluoride is preponderantly deposited in the skeleton and teeth, and the degree of skeletal storage is related to intake and age (Haynes and Murad 1985). This is thought to be a function of the turnover rate of skeletal components, with growing bone showing a greater fluoride deposition than bone in mature animals. Although both male and female rats were identical in age during this study, male rats typically grow at approximately twice the rate of females, which may explain the change in CaP observed only in male rats.

The description of thymus atrophy was a subjective judgment made during necropsy thatwas not verified by histopathologic examination. Inflammation and hemorrhage of the deglutitory organs can be attributed to the overall debilitated condition of the rats, which resulted in increased difficulty performing the daily gavage treatment. The gavage trauma produced chronic esophagitis, which resulted in lymphocytosis and hyperplastic mesentery and mandibular lymph nodes. Hepatocellular swelling was considered to be a distinct, treatment-related finding in this study and was persistent after the 14-day posttreatment period. The cytoplasmic changes may have included proliferation of smooth endoplasmic reticulum and/or peroxisomes. Studies designed to assess the ultrastructural bases for the hepatocytic lesions and to compare hepatic effects of HC 27-S with other compounds believed to cause similar hepatocytic effects are in progress. It was also apparent that adrenocortical vacuolar degeneration was a treatment-related effect that remained unreversed following the 14-day posttreatment period. Although increased kidney weights were noted in all treatment groups, no necrosis of the *pars recta* of the proximal tubules or degenerative changes were found as have been reported in rats exposed to CTFE monomer (Buckley *et al.* 1982).

In summary, this study indicated that repeat oral administration of HC 27-S results in toxic effects not unlike those of acute fluoride poisoning. Although HC 27-S is a polymer of CTFE, the

overall toxic effects were not typical of those observed following intoxication with CTFE or with shorter oligomers of CTFE. Signs of nephrotoxicity, diuresis, and increased water consumption were not evident. Urine-concentrating ability was unaffected in both sexes of rats. Defluorination of the HC 27-S oligomer, as indicated by increased urine fluoride levels and changes in CaP ratios in the femurs of male rats, was evident throughout the study. Gross liver enlargement and microscopic hepatocellular cytomegaly are findings that indicate that the liver is probably the primary rat organ injured by the repeated oral doses of HC 27-S used in this study.

SECTION 5

REFERENCES

Barcikowski, R.S. 1983. Computer Packages and Research Design. Vol. 1: BMDP, Lanham, MD: University Press of America.

Buckley, L.A., J.W. Clayton, R.B. Nagle, and A.J. Gandolfi. 1982. Chlorotrifluoroethylene nephrotoxicity in rats: a subacute study. *Fund. Appl. Toxicol.* 2:181-186.

Clayton, J. W. 1977. Toxicology of fluoroalkenes: review and research needs. *Environ. Health Perspect.* 21:255.

Dixon, W.J. 1985. *BMDP Statistical Software*. Berkeley, CA: University of California Press.

Guyton, A.C. 1976. *Textbook of Medical Physiology*. Philadelphia, PA: W.B. Saunders Company, pp. 973-981.

Haynes, R.C., Jr. and F. Murad. 1985. Agents Affecting Calcification: Calcium, Parathyroid Hormone, Calcitonin, Vitamin D, and Other Compounds. In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics.* New York, NY: MacMillan Publishing Company, 7th Edition, pp. 1517-1540.

Jones, T.C. and R.D. Hunt. 1983. *Veterinary Pathology.* Philadelphia, PA: Lea and Feibiger, pp. 972-978.

Jubb, K.V., P.D. Kennedy, and N. Palmer. 1985. *Pathology of Domestic Animals.* Vol 1. Orlando, FL: Academic Press, Inc., pp. 54-58.

Kinkead, E.R., C.L. Gaworski, J.R. Horton, and T.R. Boosinger. 1987. Chlorotrifluoroethylene Oligomer: Evaluation of Acute Delayed Neurotoxicity in Hens, and Study of Absorption and Metabolism in Rats Following Oral, Dermal, and Inhalation Exposure. (No. AAMRL-TR-87-044.) Wright-Patterson Air Force Base, OH: Armstrong Aerospace Medical Research Laboratory.

Neefus, J.D., J. Cholak, and B.E. Saltzman. 1970. The determination of fluoride in urine using a fluoride-specific ion electrode. *Am. Ind. Hyg. Assoc. J.* 31:96-99.

Patty, F.A. (Ed.) 1963. Industrial Hygiene and Toxicology. 2nd Edition: New York, Wiley and Sons, Inc., pp. 835-841.

Potter, C.L., A.J. Gandolfi, R. Nagle, and J.W. Clayton. 1981. Effects of inhaled chlorotrifluoroethylene and hexafluoropropene on the rat kidney. *Toxicol. Appl. Pharmacol.* 59:431-440.

Singer, L. and R.H. Ophaug. 1979. Concentrations of ionic, total, and bound fluoride in plasma. *Clin. Chem.* 25(4):523-525.

Widmann, F.K. 1973. Goodale's Clinical Interpretation of Laboratory Tests. 7th Ed. Philadelphia, PA: F.A. Davis Company.

Zar, J.H. 1974. Biostatistical Analysis. Englewood Cliffs, NJ: Prentice Hall.

SECTION 6

QUALITY ASSURANCE

The study "The Determination of the Acute and Repeated Oral Toxicity of Halocarbon Oil, Series 27-S" was conducted by Northrop Services, Inc. – Environmental Sciences, Toxic Hazards Research Unit in compliance with the Environmental Protection Agency's Good Laboratory Practices Guidelines, 40CFR PART 792. The various phases of this study were inspected by members of the Quality Assurance Group. Results of these inspections were reported directly to the Study Director at the close of each inspection.

DATE OF INSPECTION:	ITEM INSPECTED:
16 March 1987	Oral dosing
17 March 1987	Specimen collection and analysis for fluoride Ion
10-16 December 1987	Records inventory and data audit
21 December 1987	Data audit
29 December 1987	Draft report review

The Quality Assurance Group has determined by review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretation presented in this Final Report.

M. G. Schneider Jr

M. G. Schneider
QA Coordinator
Northrop Services, Inc.
Environmental Sciences
Toxic Hazards Research Unit

Date 3 January 1989

APPENDIX A

BODY WEIGHTS (g) OF F-344 RATS FOLLOWING SINGLE ORAL GAVAGE OF HC 27-S

TABLE A.1

Male Rats

Animal No.	Day 0	Day 1	Day 4	Day 7	Day 10	Day 14
01400002	178	189	202	212	219	226
01400021	188	204	210	222	229	240
01400023	169	181	190	205	209	224
01400026	182	198	208	223	228	233
01400027	166	179	190	204	209	217
Mean	177	190	200	213	219	228
SEM	4.1	4.8	4.4	4.1	4.3	4.0

Female Rats

Animal No.	Day 0	Day 1	Day 4	Day 7	Day 10	Day 14
01400035	129	138	141	146	148	157
01400040	130	143	147	149	146	156
01400041	135	142	149	151	152	158
01400047	133	143	149	147	151	157
01400051	131	143	144	143	149	154
Mean	131	142	146	147	149	156
SEM	1.0	1.0	1.6	1.3	1.0	0.8

APPENDIX B

MEAN WHOLE BLOOD PARAMETERS FOR MALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

TABLE 8.1

	7 Days	21 Days	35 Days
WBC (x 10^3 cells/mm^3)			
Control	5.75 ± 0.30	5.37 ± 0.31	8.88 ± 0.50
Test	6.74 ± 0.30[b]	6.66 ± 0.44[c]	8.01 ± 0.56
RBC (x 10^6 cells/mm^3)			
Control	8.32 ± 0.10	8.17 ± 0.06	7.77 ± 0.32
Test	7.66 ± 0.19	7.42 ± 0.13	7.00 ± 0.36
HGB (g/dL)			
Control	16.25 ± 0.19	16.05 ± 0.12	15.25 ± 0.39
Test	15.48 ± 0.39	14.38 ± 0.20	13.53 ± 0.58
HCT (%)			
Control	42.70 ± 1.20	42.62 ± 0.31	40.88 ± 1.60
Test	42.00 ± 1.71	39.32 ± 0.37	36.13 ± 2.21
MCV (mm^3)			
Control	53.12 ± 0.32	52.10 ± 0.27	52.55 ± 0.21
Test	53.22 ± 0.40	52.30 ± 0.39	51.85 ± 0.48
MCH (10^{-12}/cells/mm^3)			
Control	19.50 ± 0.18	19.63 ± 0.03	19.70 ± 0.52
Test	20.22 ± 0.12	19.38 ± 0.16	19.68 ± 1.52
MCHC (%)			
Control	36.70 ± 0.53	37.63 ± 0.14	37.40 ± 0.95
Test	38.00 ± 0.22	37.04 ± 0.03	31.50 ± 6.60
Neutrophils (%)			
Control	19.00 ± 2.35	31.83 ± 2.70	25.75 ± 4.15
Test	19.40 ± 2.21	20.20 ± 2.82[b]	13.00 ± 2.27[b]
Lymphocytes (%)			
Control	75.83 ± 2.17	64.33 ± 3.58	65.25 ± 4.77
Test	74.00 ± 2.41	75.20 ± 2.71[c]	79.75 ± 4.44[b]
Monocytes (%)			
Control	3.50 ± 0.99	5.00 ± 1.08	5.75 ± 1.32
Test	6.20 ± 0.97	4.60 ± 0.68	5.50 ± 2.02
Eosinophils (%)			
Control	1.40 ± 0.25	1.00 ± 0.00	1.50 ± 0.50
Test	1.00 ± 0.00	0.00 ± 0.00	2.00 ± 1.00

[a] Mean ± SEM
[b] Statistically different from control $p<0.01$.
[c] Statistically different from control $p<0.05$.

APPENDIX C

MEAN WHOLE BLOOD PARAMETERS FOR FEMALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S

TABLE C.1

	7 Days	21 Days	35 Days
WBC (x 10^3 cells/mm^3)			
Control	5.78 ± 0.25	8.10 ± 0.38	7.72 ± 0.69
Test	8.70 ± 0.35[b]	9.43 ± 0.77[c]	6.82 ± 0.49
RBC (x 10^6 cells/mm^3)			
Control	7.95 ± 0.13	6.86 ± 0.24	7.49 ± 0.09
Test	7.56 ± 0.15	7.01 ± 0.14	7.03 ± 0.18
HGB (g/dL)			
Control	16.17 ± 0.25	14.96 ± 0.32	15.42 ± 0.16
Test	15.68 ± 0.24	14.10 ± 0.36	14.50 ± 0.34
HCT (%)			
Control	43.25 ± 0.63	37.00 ± 1.47	40.60 ± 0.57
Test	41.36 ± 0.76	37.97 ± 0.63	39.12 ± 1.04
MCV (mm^3)			
Control	54.33 ± 0.15	53.88 ± 0.55	54.13 ± 0.34
Test	54.60 ± 0.28	54.17 ± 0.58	55.58 ± 0.22
MCH (10^{-12}/cells/mm^3)			
Control	20.32 ± 0.23	21.90 ± 0.91	20.58 ± 0.07
Test	20.72 ± 0.12	20.10 ± 0.15	20.58 ± 0.07
MCHC (%)			
Control	37.37 ± 0.32	40.72 ± 1.87	37.98 ± 0.21
Test	37.94 ± 0.27	37.10 ± 0.67	37.00 ± 0.21
Neutrophils (%)			
Control	17.83 ± 0.65	15.80 ± 2.62	17.67 ± 2.54
Test	17.00 ± 3.13	10.33 ± 0.88[b]	13.60 ± 0.51[b]
Lymphocytes (%)			
Control	79.00 ± 0.68	80.40 ± 2.89	80.50 ± 2.14
Test	78.20 ± 3.34	86.00 ± 1.53[c]	84.40 ± 0.93[b]
Monocytes (%)			
Control	2.17 ± 0.48	3.00 ± 0.84	1.75 ± 0.48
Test	3.00 ± 0.55	2.00 ± 0.58	1.75 ± 0.25
Eosinophils (%)			
Control	1.00 ± 0.00	1.33 ± 0.33	1.00 ± 0.00
Test	1.00 ± 0.00	1.00 ± 0.00	1.67 ± 0.33

[a] Mean ± SEM
[b] Statistically different from control $p<0.01$.
[c] Statistically different from control $p<0.05$.

APPENDIX D

CAP RATIO OF FEMURS FROM MALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S FOR 7 DAYS

TABLE D.1

Control Animal #	Spectra[a]	Ca	P	CaP	Mean[b]	Group Mean[c]
1	A	42.13	28.19	1.49		
	B	42.44	27.42	1.55		
	C	43.76	28.29	1.55	1.58 ± .02	
	D	47.82	29.99	1.59		
	E	49.06	29.58	1.66		
	F	47.95	29.61	1.62		
2	A	52.12	30.73	1.70		
	B	53.41	30.42	1.76		
	C	53.81	30.93	1.74	1.75 ± .01	1.63 ± .02
	D	55.68	31.11	1.79		
	E	55.13	31.27	1.76		
	F	55.44	31.40	1.77		
3	A	39.35	25.35	1.55		
	B	44.99	28.92	1.56		
	C	46.83	29.90	1.57	1.57 ± .01	
	D	44.53	28.82	1.55		
	E	46.08	29.44	1.57		
	F	48.52	30.14	1.61		
Treated Animal #						
1	A	56.74	27.19	2.09		
	B	58.50	27.42	2.13		
	C	53.27	26.98	1.97	2.03 ± .03	
	D	53.26	26.98	1.97		
	E	53.43	27.91	1.91		
	F	56.77	27.44	2.07		
2	A	57.15	28.36	2.02		
	B	56.76	28.53	1.99		
	C	61.13	27.89	2.19	2.05 ± .03	2.05 ± .02[d]
	D	57.74	28.16	2.05		
	E	59.31	29.47	2.01		
	F	59.25	29.21	2.03		
3	A	60.31	30.00	2.01		
	B	61.45	29.33	2.10		
	C	59.98	28.31	2.12	2.08 ± .02	
	D	59.30	28.85	2.06		
	E	58.64	28.71	2.04		
	F	61.49	28.62	2.15		

a Spectra were analyzed at six sites per femur.
b Mean ± SEM (N = 6).
c Mean ± SEM (N = 18).
d Statistically different from control $p<0.01$.

APPENDIX E

CAP RATIO OF FEMURS FROM FEMALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S FOR 7 DAYS

TABLE E.1

Control Animal #	Spectra[a]	Ca	P	CaP	Mean[b]	Group Mean[c]
1	A	41.88	26.89	1.56		
	B	40.76	27.21	1.50		
	C	45.28	28.62	1.58	1.53 ± .01	
	D	41.98	28.02	1.50		
	E	42.43	28.12	1.51		
	F	42.74	27.65	1.55		
2	A	44.48	27.07	1.64		
	B	44.01	27.98	1.57		
	C	45.98	29.31	1.57	1.61 ± .01	1.63 ± .02
	D	47.13	29.07	1.62		
	E	47.69	29.02	1.64		
	F	47.47	29.21	1.63		
3	A	50.24	29.15	1.72		
	B	49.00	28.80	1.70		
	C	47.33	28.61	1.65	1.74 ± .02	
	D	52.97	29.53	1.79		
	E	52.58	29.43	1.79		
	F	52.42	29.20	1.80		
Treated Animal #						
1	A	49.17	27.23	1.81		
	B	49.18	27.16	1.81		
	C	44.59	25.60	1.74	1.79 ± .01	
	D	46.59	26.19	1.78		
	E	47.74	25.64	1.86		
	F	45.42	25.63	1.77		
2	A	47.30	28.85	1.64		
	B	47.59	29.01	1.64		
	C	48.42	29.44	1.64	1.60 ± .02	1.66 ± .03
	D	43.24	27.96	1.55		
	E	43.67	28.32	1.54		
	F	44.23	28.33	1.56		
3	A	42.22	27.68	1.53		
	B	41.21	27.90	1.48		
	C	41.21	27.87	1.48	1.51 ± .01	
	D	42.11	27.77	1.52		
	E	42.09	27.71	1.52		
	F	42.73	27.73	1.54		

a Spectra were analyzed at six sites per femur.
b Mean ± SEM (N = 6).
c Mean ± SEM (N = 18).

APPENDIX F

CAP RATIO OF FEMURS FROM MALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S FOR 21 DAYS

TABLE F.1

Control Animal #	Spectra[a]	Ca	P	CaP	Mean[b]	Group Mean[c]
1	A	45.29	28.98	1.56		
	B	41.75	27.82	1.50		
	C	42.02	27.90	1.51	1.58 ± .02	
	D	49.45	30.26	1.63		
	E	48.89	29.76	1.64		
	F	48.56	30.05	1.62		
2	A	53.21	29.98	1.77		
	B	51.50	30.28	1.70		
	C	52.01	29.99	1.73	1.70 ± .02	1.64 ± .02
	D	50.06	29.07	1.72		
	E	48.81	30.01	1.63		
	F	47.62	29.54	1.61		
3	A	51.34	30.53	1.68		
	B	48.36	29.40	1.64		
	C	45.45	28.61	1.59	1.64 ± .01	
	D	52.12	30.97	1.68		
	E	51.54	31.29	1.65		
	F	49.53	30.56	1.62		
Treated Animal #						
1	A	53.22	25.59	2.08		
	B	54.65	26.37	2.07		
	C	56.37	28.38	1.99	1.93 ± .05	
	D	50.83	27.98	1.82		
	E	50.44	27.65	1.82		
	F	49.86	27.32	1.83		
2	A	60.88	28.60	2.13		
	B	59.10	30.20	1.96		
	C	59.55	30.29	1.97	1.98 ± .03	1.96 ± .02[d]
	D	55.28	28.37	1.95		
	E	55.16	29.15	1.89		
	F	56.93	28.62	1.99		
3	A	49.10	25.59	1.92		
	B	53.17	24.94	2.13		
	C	47.77	23.76	2.01	1.97 ± .03	
	D	43.51	23.23	1.87		
	E	44.37	22.56	1.97		
	F	44.84	23.14	1.94		

a Spectra were analyzed at six sites per femur.
b Mean ± SEM (N = 6).
c Mean ± SEM (N = 18).
d Statistically different from control $p < 0.01$.

APPENDIX G

CAP RATIO OF FEMURS FROM FEMALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S FOR 21 DAYS

TABLE G.1

Control Animal #	Spectra[a]	Ca	P	CaP	Mean[b]	Group Mean[c]
1	A	46.07	28.01	1.64		
	B	45.74	27.60	1.66		
	C	44.94	27.23	1.65	1.63 ± .01	
	D	45.59	27.83	1.64		
	E	46.14	28.28	1.63		
	F	42.36	27.23	1.56		
2	A	41.67	27.44	1.52		
	B	41.92	27.35	1.53		
	C	42.39	27.71	1.53	1.57 ± .02	1.59 ± .01
	D	47.35	29.27	1.62		
	E	47.28	29.75	1.59		
	F	48.09	29.75	1.62		
3	A	41.46	27.13	1.53		
	B	42.00	27.16	1.55		
	C	43.49	27.71	1.57	1.58 ± .02	
	D	44.94	28.58	1.57		
	E	45.33	28.44	1.59		
	F	47.44	28.85	1.64		
Treated Animal #						
1	A	41.60	26.71	1.56		
	B	41.58	26.78	1.55		
	C	43.31	26.90	1.61	1.61 ± .02	
	D	46.85	28.51	1.64		
	E	47.15	28.95	1.63		
	F	49.15	29.05	1.69		
2	A	49.91	29.54	1.69		
	B	48.63	29.54	1.65		
	C	48.53	28.82	1.68	1.64 ± .01	1.66 ± .02
	D	44.76	27.91	1.60		
	E	45.34	28.38	1.60		
	F	46.36	28.63	1.62		
3	A	45.80	28.14	1.63		
	B	46.25	28.48	1.62		
	C	51.34	29.11	1.76	1.71 ± .03	
	D	52.07	29.16	1.79		
	E	52.14	29.75	1.75		
	F	51.36	29.58	1.74		

a Spectra were analyzed at six sites per femur.
b Mean ± SEM (N = 6).
c Mean ± SEM (N = 18).

APPENDIX H

CAP RATIO OF FEMURS FROM MALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S FOR 21 DAYS AND HELD 14 DAYS

TABLE H.1

Control Animal #	Spectra[a]	Ca	P	CaP	Mean[b]	Group Mean[c]
1	A	48.72	30.36	1.60		
	B	48.56	29.94	1.62		
	C	48.75	30.24	1.61	1.62 ± .01	
	D	48.50	29.62	1.64		
	E	48.51	29.77	1.63		
	F	48.90	29.78	1.64		
2	A	35.75	26.09	1.37		
	B	36.36	26.52	1.37		
	C	38.82	27.21	1.43	1.35 ± .02	1.55 ± .03
	D	34.92	26.18	1.33		
	E	32.93	25.22	1.31		
	F	32.43	25.26	1.28		
3	A	46.15	27.48	1.68		
	B	44.42	27.05	1.64		
	C	45.76	27.32	1.67	1.66 ± .01	
	D	46.69	27.91	1.67		
	E	45.65	27.47	1.66		
	F	44.53	27.06	1.65		
Treated Animal #						
1	A	60.35	32.25	1.87		
	B	57.95	32.36	1.79		
	C	57.70	32.43	1.78	1.79 ± .02	
	D	57.28	32.36	1.77		
	E	57.39	32.40	1.77		
	F	57.43	32.93	1.74		
2	A	62.06	28.54	2.17		
	B	61.87	28.97	2.14		
	C	62.40	28.26	2.21	2.18 ± .03	1.87 ± .06[d]
	D	62.09	26.94	2.30		
	E	61.49	28.88	2.13		
	F	61.42	28.72	2.14		
3	A	48.16	29.23	1.65		
	B	47.69	29.32	1.63		
	C	46.53	28.58	1.63	1.64 ± .01	
	D	48.71	29.35	1.66		
	E	48.57	29.85	1.63		
	F	48.73	29.82	1.63		

a Spectra were analyzed at six sites per femur.
b Mean ± SEM (N = 6).
c Mean ± SEM (N = 18).
d Statistically different from control $p<0.01$.

APPENDIX I

CAP RATIO OF FEMURS FROM FEMALE F-344 RATS FOLLOWING REPEATED ORAL ADMINISTRATION OF HC 27-S FOR 21 DAYS AND HELD 14 DAYS

TABLE I.1

Control Animal #	Spectra[a]	Ca	P	CaP	Mean[b]	Group Mean[c]
1	A	51.83	31.44	1.65		
	B	53.73	31.92	1.68		
	C	51.70	31.04	1.67	1.67 ± .01	
	D	50.62	30.13	1.68		
	E	50.15	29.98	1.67		
	F	50.74	29.98	1.69		
2	A	50.42	29.76	1.69		
	B	50.10	29.33	1.71		
	C	49.80	29.70	1.68	1.71 ± .01	1.68 ± .01
	D	52.00	30.12	1.73		
	E	51.99	30.09	1.73		
	F	51.01	29.70	1.72		
3	A	49.17	30.64	1.60		
	B	51.96	31.07	1.67		
	C	50.21	31.27	1.61	1.65 ± .01	
	D	50.64	30.46	1.66		
	E	50.89	30.42	1.67		
	F	50.93	30.45	1.67		
Treated Animal #						
1	A	55.14	29.61	1.86		
	B	55.05	29.60	1.86		
	C	54.92	30.22	1.82	1.83 ± .01	
	D	55.84	30.78	1.81		
	E	55.78	31.22	1.79		
	F	56.27	30.88	1.82		
2	A	47.26	27.24	1.73		
	B	45.46	28.38	1.60		
	C	45.05	28.28	1.59	1.64 ± .02	1.68 ± .03
	D	50.03	30.23	1.65		
	E	50.57	30.31	1.67		
	F	48.32	30.29	1.60		
3	A	45.92	29.20	1.57		
	B	44.73	28.75	1.56		
	C	44.90	28.60	1.57	1.57 ± .01	
	D	43.85	28.39	1.54		
	E	44.50	28.85	1.54		
	F	46.36	28.63	1.62		

a Spectra were analyzed at six sites per femur.
b Mean ± SEM (N = 6).
c Mean ± SEM (N = 18).

* U.S. GOVERNMENT PRINTING OFFICE: 1988 - 668-002/80238